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PHARMACODYNAMICS

Reversal of mecamylamine-induced effects in healthy subjects by nicotine receptor agonists: Cognitive and (electro) physiological responses

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AIMS

Establishing a pharmacological challenge model could yield an important tool to understand the complex role of the nicotinic cholinergic system in cognition and to develop novel compounds acting on the nicotinic acetylcholine receptor.

METHODS

This randomized, double-blind, double-dummy, placebo-controlled, four-way crossover study examined the effects of the nicotinic antagonist mecamylamine on a battery of cognitive and neurophysiological test with coadministration of a placebo, nicotine or galantamine in order to reverse the cognitive impairment caused by mecamylamine.

RESULTS

Thirty-three healthy subjects received a single oral dose of 30 mg of mecamylamine (or placebo) in combination with either 16 mg of oral galantamine or 21 mg of transdermal nicotine (or its double-dummy). Mecamylamine 30 mg induced significant disturbances of cognitive functions. Attention and execution of visual (fine) motor tasks was decreased, short- and long-term memory was impaired and the reaction velocity during the test was slower when compared to placebo. Mecamylamine 30 mg produced a decrease in posterior α and β power in the surface electroencephalogram, effects that were reversed by nicotine co-administration. Memory and motor coordination tests could be partially reversed by the coadministration of nicotine.

CONCLUSIONS

Mecamylamine administration induced slowing of the electroencephalogram and produced decrease in performance of tests evaluating motor coordination, sustained attention and short- and long-term memory. These effects could be partially reversed by the coadministration of nicotine, and to a lesser extent by galantamine.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Mecamylamine, a nicotinic antagonist, induces symptoms resembling those of Alzheimer's disease in healthy subjects. Mecamylamine effects in humans have so far only been reversed in preclinical experimental models.

WHAT THIS STUDY ADDS

- Acute administration of mecamylamine 30 mg in humans induces a significant disturbance in cognitive functions such as visuo-motor coordination, short- and long-term memory, reaction time, and decrease in α and β power in the electroencephalogram. Coadministration of nicotine led to significant reversal of some of the mecamylamine induced cognitive deficits.

Introduction

The cholinergic system plays an important role in key cognitive processes such as attention and working and associative memory, and is considered essential for learning [1, 2]. Cholinergic dysfunction is recognized to be involved in the pathophysiology of neurodegenerative diseases [e.g. Alzheimer's disease (AD), Parkinson's disease] and psychiatric conditions (e.g. schizophrenia) and is therefore considered a promising therapeutic target [3, 4].

Scopolamine, a competitive muscarinic antagonist, is the most frequently used challenge drug to induce temporary, reversible, cognitive disturbances resembling those of AD in healthy subjects [5, 6]. Challenging the healthy system to induce disease-like symptoms is important in early proof-of-pharmacology of new drugs. With multiple nicotinic receptor agonists in the clinical phase of drug development [7, 8], the interest in nicotinic acetylcholine receptor (**nAChR**) pharmacology is rising. The use of the muscarinic receptor antagonist scopolamine to investigate the pharmacology of nicotinic drugs is, in our view, inappropriate and less direct, and therefore we aimed to develop a pharmacological challenge targeting the nicotinic cholinergic system.

Mecamylamine is a nonselective noncompetitive nAChR antagonist [9]. Mecamylamine 20 mg produced impairments in learning and retrieval [10], acquisition, increased reaction time and errors [11] and an increased inspection time during a visual discrimination test [12] in healthy subjects, cognitive deficits that are also observed in patients with AD. To be able to use mecamylamine as a challenge model to prove pharmacological effects of nicotinic compounds, it is necessary to demonstrate reversal of its temporary negative effects on cognition. In animals, successful reversal of mecamylamine-induced disturbances was demonstrated with **nicotine** coadministration [13, 14]. To our knowledge, only one study in humans described partial reversal of increased inspection time induced by 20 mg of mecamylamine, when 5 mg of donepezil, an acetylcholinesterase inhibitor, was coadministered [12].

In a previous exploratory study, we confirmed that administration of 10 and 20 mg of mecamylamine in healthy subjects led to a temporary, dose-dependent disturbance of several cognitive functions including fine motor coordination and fluency, short- and long-term memory, attention, and concentration [15]. In this study we further investigated the dose–effect relationship of mecamylamine with a higher dose of 30 mg. Furthermore, we aimed to further validate mecamylamine as a nicotinic anticholinergic challenge by investigating the potential reversal of the observed cognitive effects of mecamylamine by coadministering **galantamine** (a cholinesterase inhibitor) and nicotine (a nAChR agonist).

Materials and methods

Study design

This was a randomized, double-blind, double-dummy, placebo-controlled, four-way cross-over study of a single oral dose of mecamylamine (or placebo) in combination with either galantamine or nicotine. The treatment arms were: mecamylamine plus placebo, mecamylamine plus nicotine, mecamylamine plus galantamine and (double) placebo. A minimal wash-out period of 1 week was implemented as the calculated terminal life of a single administration of mecamylamine was 8–11 h, nicotine 2–3 h and galantamine 7–8 h.

Oral medication was administered with water at time point zero of every visit. Five min thereafter, a nicotine or placebo patch was placed on the skin at the shoulder blade region. Subjects were discharged 32 h postdose after monitoring of vital signs was performed and if all symptoms related to study drugs disappeared.

Subject selection

A medical ethics committee approved the study protocol. After giving written informed consent, all subjects were medically screened prior to study participation. Healthy male incidental smokers (age between 18 and 45 years and body mass index between 18 and 32 kg m^{-2}, both inclusive) were included in the study. Incidental smokers, defined as subjects smoking at least once a month, but no more than five cigarettes per day, within the past 3 months, were included in the study because nonsmokers might have experienced more severe side effects derived from the nicotine and galantamine administration. Main exclusion criteria included any relevant medical abnormalities including conditions causing cognitive impairment, orthostatic hypotension [16] or hypertension (>140/90 mmHg). Use of agents or drugs known to influence CNS performance were not allowed during study participation.

Medicinal products and dosing rational

Drug accountability of all medicinal products was managed by the Leiden University Medical Centre Clinical Trials pharmacy. A full treatment description per group can be found in Table S2 of the Supporting Information.

Mecamylamine 30 mg (Euticals SpA, Milan, Italy) capsules containing 36.6 mg mecamylamine HCl and microcrystalline

cellulose as filling agent (also used in the placebo capsules) were administered orally. Based on an interim pharmacokinetic–pharmacodynamic modelling of the concentration–effect relationship of mecamylamine 10 and 20 mg on blood pressure, which was investigated in the exploratory study (data not presented), a single oral dose of 30 mg was considered safe. Moreover, the dose was expected not to exceed E_{MAX} (i.e. still allowing reversal) and not to cause functionally limiting hypotension.

Transdermal patches containing 21 mg nicotine (NiQuitin; GlaxoSmithkline, Bolton, UK), with blinding covering were applied to reverse mecamylamine effects. Blinded Vaseline patches were used as placebo. Nicotine 21 mg patches are the highest commercially available dose that is well tolerated without significant adverse events (AEs) in smokers [17].

Four galantamine hydrobromide 4 mg over-encapsulated capsules (Reminyl; Janssen-Cilag SpA, Latina, Italy) or matching placebo capsules were administered orally, for a total dose of 16 mg. Lactose monohydrate 125 mg tablets were used as placebo. Single doses up to 15 mg without titration have been safely administered in healthy subjects [18] and, in our centre, galantamine 16 mg was previously administered in healthy elderly subjects (unpublished data) and found to be safe. Galantamine was chosen as it exerts an allosteric nicotinic modulatory activity next to the cholinesterase inhibitory effect, which donepezil lacks *in vitro* [19–21].

Cognitive and neurophysiology measurements

The NeuroCart is a computerized test-battery of sensitive tests used to evaluate a wide range of central nervous system (CNS) effects of neuro- and psychoactive drugs. A practice session for all tests was performed at screening for test familiarization. At each study visit, baseline training was performed twice to ensure stable performance and minimize learning effects. The NeuroCart test battery was subsequently performed at time points 30, 80, 130, 180, 230, 280, 360 and 480 min postdose, except for the visual verbal learning test (VVLT), which was only performed once per occasion, and the Milner maze test (MMT), which was not performed at 130 and 230 min.

N-back test. Subjects were asked to remember and correlate a sequence of letters presented in a random order [22] thereby evaluating (short-term) working memory as participants should match, encode and response to the order of consonants in the test. The N-back test consists of three conditions, with increased working memory load. Letters were presented consecutively on the screen with a speed of 30 letters min^{-1}. In the first condition subjects had to indicate whether the letter on the screen was an "X". In the second condition, subjects indicated whether the letter seen was identical to the previous letter. In the third condition, subjects were asked to indicate whether the letter was identical to two letters before the letter seen. Performance is expressed as the ratio of correct and incorrect answers [(correct – incorrect) × $total^{-1}$] and reaction time on the 0-, 1- and 2-back conditions.

Adaptive tracking test. The test is a pursuit-tracking task, measuring attention and eye-hand coordination [23, 24]. A circle moves pseudo-randomly about a screen. The subject is instructed to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance scores over a period of time of 3 min were used for analysis.

EEG. Resting state eyes-closed EEG recordings were obtained for 64 s per time point using four cranial superficial gold electrodes (Fz, Cz, Pz, Oz), placed following the 10–20 system and fixed with EC2 paste with the same common ground and eye movement registration. Electrode resistance was kept below 5 kΩ. The grass 15LT series Amplifier Systems was used for signal amplification with a time constant of 0.3 s and a low pass filter at 100 Hz. The signal was AD-converted using CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Fast Fourier transformed absolute power (μV) was calculated from the raw measurements in the α [7.5–13.5 Hz], β [13.5–35 Hz], δ [2–4 Hz], θ [4–7.5 Hz] and γ [>35 Hz] frequency ranges in two bipolar leads: Fz-Cz and Pz-Oz.

Finger tapping. The dominant hand finger tapping test was performed to evaluate motor activation and fluency [25, 26]. The volunteer was instructed to tap as quickly as possible with the index finger of the dominant hand. Each session contained five performances of 10 s. The mean tapping rate of five trials per time point was used for statistical analysis.

Simple reaction time test. The test measures the attention and speed of information processing of the participant. At random intervals (0.5–1.5 s), a white circle appears in the centre of a black computer screen. Participants were instructed to press the space bar with the index finger of their dominant hand each time the circle appears. They were instructed to respond as quickly as possible after appearance of the circle. A total of 40 circles were presented per timepoint, and the duration of the task was approximately 1 min. The outcome of the task is the time between stimulus display and response [27].

VVLT. This test evaluates the different aspects of learning (i.e. acquisition, consolidation, storage, retrieval) [26, 28, 29]. Subjects were presented 30 words in three consecutive word trials. Each trial ended with a free recall of the presented words (Immediate Recall). Approximately 30 min after start of the first trial, the volunteers were asked to recall as many words as possible (Delayed Recall). Immediately thereafter, the volunteers underwent memory recognition test, which consisted of 15 words previously presented and 15 'distractors' (Recognition).

MMT. The MMT is a visuospatial working memory test [30]. Participants were asked to find a 28-step hidden maze pathway concealed in a computer tile grid beginning at the top left corner. Participants must follow several rules: diagonal moves, backward, or more than one tile at a time was not allowed. The computerized version has five immediate, one delayed and one reverse trials where the

same maze has to be completed in the reverse order. Outcome measures are time to complete (milliseconds) and accuracy (number correct and incorrect steps) per trial.

Visual analogue scales. The visual analogues scale (VAS) is frequently used to measure subjective feelings of drug effects, as previously described [31]. From these measurements, three main factors are calculated as described by the authors: alertness (from nine scores), contentedness (often called mood; from five scores), and calmness (from two scores). A VAS evaluating nausea was also applied.

Pupil diameter measurements. Pupil diameter was determined using a digital camera (Canon Powershot A620). The subject was instructed to look into the lens. A picture of the eyes was taken using a camera with flash. All pictures were stored digitally. The diameters of the pupil and the iris were determined in the number of pixels used horizontally. For each eye, these values were recorded on data collection forms, and the pupil/iris ratio was subsequently calculated as a measure of pupil size [26, 32].

Physiological measures

Safety assessments, including registration of AEs, electrocardiogram, body temperature, blood pressure and heart rate were performed at predefined times throughout the study. Haematology, biochemistry, urinalysis, alcohol and drugs test were performed at medical screening, predose per visit and at follow-up.

Statistical analysis

The randomization scheme was elaborated prior to inclusion. Subjects were allocated to each trial group to balance the allocation. All variables were summarized by treatment and time. Repeatedly measured data were analysed with a mixed model analysis of variance with fixed factors treatment, session number (i.e. occasions 1–4), time (in min with the dosing time as reference) and treatment-by-time and as random factors subject, subject-by-treatment and subject-by-time and the average predose values as covariate. Single measured pharmacodynamic data were compared with a mixed model analysis of variance with fixed factors treatment, session number, random factors subject and the average predose values as covariate. No adjustment for multiple comparisons was performed since the main aim of the study was demonstrate a generalized trend of reversal and to obtain an impression of the magnitude of pharmacodynamic effects that can be expected from a full nicotinic receptor agonist. The analysis was performed by an independent statistician using SAS software for windows v9.4 (SAS Institute, Inc., Cary, NC, USA). Graphs were created using R v2.14.1 (R Foundation for Statistical Computing, Vienna, Austria).

Sample size determination

Sample size calculations were performed using the data obtained from the recall parameter from the VVLT, performed in the previous study with mecamylamine 10 and 20 mg compared to placebo. The sample size was calculated using 80% power in a paired *t*-test with a two-sided 0.05 significance level with a mean difference of 3.8, assuming a standard deviation of differences of 4.30.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [33], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [34].

Results

Subject demographics

Fifty-one healthy male subjects underwent medical screening and thirty-three subjects were included in the study. The mean age was 23.3 years (range 19–35), average body weight was 74.5 ± 8.3 kg (range 60.25–91.25) and body mass index was 22.6 ± 2.4 kg m^{-2} (range 19.4–27.7). Twenty-seven subjects completed all four study visits. Five subjects cancelled their participation after the first visit due to side effects (nausea, vomit, obstipation, fatigue and feeling abnormal). One subject was withdrawn from the study (before being randomized) because it was not possible to place an intravenous catheter and one subject stopped his participation for personal reasons.

Cognitive and neurophysiological measurements

The complete summary of the contrasts and the least squared means can be found in Table S1 of the Supporting Information.

Adaptive tracking test. The mean performance on the adaptive tracking test was significantly influenced by mecamylamine administration (overall effect $P < 0.0001$), as shown in Table 1. As expected, mecamylamine alone produced a significant impairment in the mean performance of on average – 3.3% (95% confidence interval: –4.6 to –2.0, $P < 0.0001$) in adaptive tracking performance. Coadministration of nicotine caused a significant improvement of on average 1.5% (95% confidence interval: 0.2–2. 8, $P < 0.05$; Figure 1A) in comparison to mecamylamine alone.

N-back test. Examination of the mean correct – incorrect ratio on the 0-back condition showed a significant overall effect ($P = 0.0410$), producing on average a decrease of –0.023 (95% confidence interval: –0.044 to –0.003, $P < 0.05$) in the ratio after administration of mecamylamine (Figure 2A, Table 1), reflecting a worsening in test performance.

Regarding the reaction time (RT) during the N-back test, the only paradigm where a significant overall effect ($P = 0.0432$) was observed was the 2-back, the most difficult condition. Mecamylamine administration produced a mean increase of 28.3 ms (95% confidence interval: 2.0–54.6, $P < 0.05$) on 2-back RT (Figure 1B). The increase in RT due to administration of mecamylamine was significantly reversed by the coadministration of both nicotine (mean

Table 1

Mean difference per treatment group on the neurological parameters

		Contrasts					
		Mecamylamine *vs.* placebo		Mecamylamine + galantamine *vs.* mecamylamine		Mecamylamine + nicotine *vs.* mecamylamine	
Parameter	**Treatment F-value *P*-value**	**Mean (CI)**	***P* value**	**Mean (CI)**	***P* value**	**Mean (CI)**	***P* value**
Adaptive tracking (%)	F(3, 63.8) = 10.55 $P < 0.0001$	–3.27 (–4.58 to –1.97)	**<0.0001**	0.223 (–1.09–1.537)	0.7355	1.467 (0.153–2.780)	**0.0292**
Taps: mean of five trials (n/10 s)	F(3, 70.4) = 27.4 $P < 0.0001$	–5.30 (–6.80 to –3.80)	**<0.0001**	–.586 (–2.08–0.909)	0.4367	–0.158 (–1.66–1.341)	0.8343
0-back ratio (correct – incorrect/total)	F(3, 70.5) = 2.90 $P = 0.0410$	–0.023 (–0.044 to –0.003)	**0.0270**	–0.006 (–0.027–0.015)	0.5784	0.007 (–0.013–0.028)	0.4839
0-back mean RT (ms)	F(3, 61.9) = 0.47 $P = 0.7043$	8.0 (–9.8 to 25.8)	0.3721	–4.0 (–21.8–13.9)	0.6588	–9.6 (–27.4–8.2)	0.2836
1-back ratio (correct – incorrect/total)	F(3, 72.1) = 2.58 $P = 0.0602$	–.015 (–0.038–0.008)	0.2021	–0.013 (–0.036–0.010)	0.2634	0.015 (–.009–0.038)	0.2184
1-back mean RT (ms)	F(3, 67.4) = 0.36 $P = 0.7850$	6.2 (–10.0–22.4)	0.4485	0.9 (–15.3–17.2)	0.9076	–4.5 (–20.8–11.9)	0.5873
2-back ratio (correct – incorrect/total)	F(3, 73) = 2.10 $P = 0.1079$	–.0018 (–0.049–0.014)	0.2664	–0.016 (–.048–0.015)	0.3043	0.018 (–.014–0.049)	0.2689
2-back mean RT (ms)	F(3, 63.4) = 2.87 $P = 0.0432$	28.3 (2.0–54.6)	**0.0356**	–27.2 (–53.5- -0.8)	**0.0437**	–36.0 (–62.2 to –9.7)	**0.0079**
Word recall correct 1	F(3, 74.8) = 1,57 $P = 0.2031$	–1.00 (–2.32–0.32)	0.1344	–0.19 (–1.52–1.15)	0.7806	–0.30 (–1.64–1.05)	0.6601
Word recall correct 2	F(3, 74.3) = 2.35 p = 0.0790	–1.79 (–3.39 to –0.20)	0.0281	–0.10 (–1.71–1.52)	0.9040	0.37 (–1.26–2.00)	0.6534
Word recall correct 3	F(3, 71.9) = 1.66 $P = 0.1735$	–1.29 (–2.97–0.39)	0.1314	–0.53 (–2.23–1.17)	0.5365	–0.04 (–1.76–1.68)	0.9664
Delayed word recall correct	F(3, 74.4) = 2.78 $P = 0.0470$	–1.22 (–2.89–0.45)	0.1502	–1.24 (–2.93–0.45)	0.1484	0.08 (–1.63–1.79)	0.9275
Delayed word recognition correct	F(3, 77.3) = 3.19 $P = 0.0284$	–1.87 (–3.46 to –0.28)	**0.0220**	–0.49 (–2.10–1.12)	0.5440	0.29 (–1.33–1.91)	0.7200
Delayed word recognition RT (ms)	F(3, 78.1) = 0.87 $P = 0.4593$	21.98 (–26.4–70.34)	0.3681	–12.8 (–61.7–36.12)	0.6039	15.99 (–33.4–65.40)	0.5213
EEG α Fz-Cz (uV)	F(3, 67.6) = 0.45 $P = 0.7186$	–0.6% (–7.9–7.3%)	0.8760	3.9% (–3.8–12.2%)	0.3262	3.0% (–4.7–11.2%)	0.4518
EEG α Pz-Oz (uV)	F(3, 44.2) = 4.01 $P = 0.0132$	–6.2% (–13.4–1.6%)	0.1149	6.7% (–1.6–15.6%)	0.1124	14.9% (6.0–24.6%)	**0.0012**
EEG β Fz-Cz (uV)	F(3, 68) = 1.95 $P = 0.1292$	–2.6% (–7.9–3.1%)	0.3628	–1.4% (–6.8–4.5%)	0.6362	5.3% (–0.6–11.5%)	0.0778
EEG β Pz-Oz (uV)	F(3, 75.6) = 2.83 $P = 0.0439$	–7.1% (–13.7 to –0.1%)	**0.0474**	4.5% (–2.8–12.5%)	0.2315	10.7% (2.9–19.1%)	**0.0068**
EEG δ Fz-Cz (uV)	F(3, 72.2) = 0.89 $P = 0.4487$	–1.0% (–6.3–4.6%)	0.7281	–2.7% (–8.0–2.9%)	0.3282	–2.6% (–7.8–3.0%)	0.3563
EEG δ Pz-Oz (uV)	F(3, 55.6) = 0.13 $P = 0.9445$	1.8% (–6.8–11.3%)	0.6822	–1.1% (–9.5–8.1%)	0.8062	–2.6% (–11.0–6.5%)	0.5571
EEG θ Fz-Cz (uV)	F(3, 71.8) = 1.15 $P = 0.3345$	5.7% (–0.5–12.3%)	0.0735	0.2% (–5.8–6.6%)	0.9437	–0.9% (–6.8–5.4%)	0.7755
EEG θ Pz-Oz (uV)	F(3, 79.3) = 1.18 $P = 0.3225$	8.8% (–0.8–19.2%)	0.0716	0.9% (–7.9–10.6%)	0.8385	–3.8% (–12.1–5.4%)	0.4066
VAS Alertness (mm)	F(3, 71) = 1.55 $P = 0.2088$	–1.82 (–3.61 to –0.02)	**0.0470**	–1.30 (–3.13–0.53)	0.1610	0.17 (–1.63–1.97)	0.8539
VAS Calmness (mm)	F(3, 75.2) = 1.69 $P = 0.1770$	0.85 (–0.66–2.35)	0.2641	–0.04 (–1.55–1.47)	0.9533	–0.54 (–2.10–1.02)	0.4906
VAS Mood (mm)	F(3, 76.1) = 3.87 $P = 0.0124$	0.44 (–0.73–1.60)	0.4567	–0.74 (–1.91–0.42)	0.2060	–0.73 (–1.91–0.45)	0.2199
VAS Nausea log(mm)	F(3, 62.1) = 0.58 $P = 0.6272$	0.0337 (–0.079–0.1462)	0.5526	.2787 (.1670–.3903)	**<0.0001**	0.1860 (0.0746–0.2974)	**0.0013**

$P < 0.05$ values are presented bold. F(NumDF, DenDF) = F-value.

Mecamylamine *vs.* placebo = [LSM mecamylamine] – [LSM placebo]; Mecamylamine + Galantamine *vs.* Mecamylamine = [LSM mecamylamine plus galantamine] – [LSM mecamylamine]; Mecamylamine + nicotine *vs.* mecamylamine = [LSM mecamylamine plus nicotine] – [LSM mecamylamine].

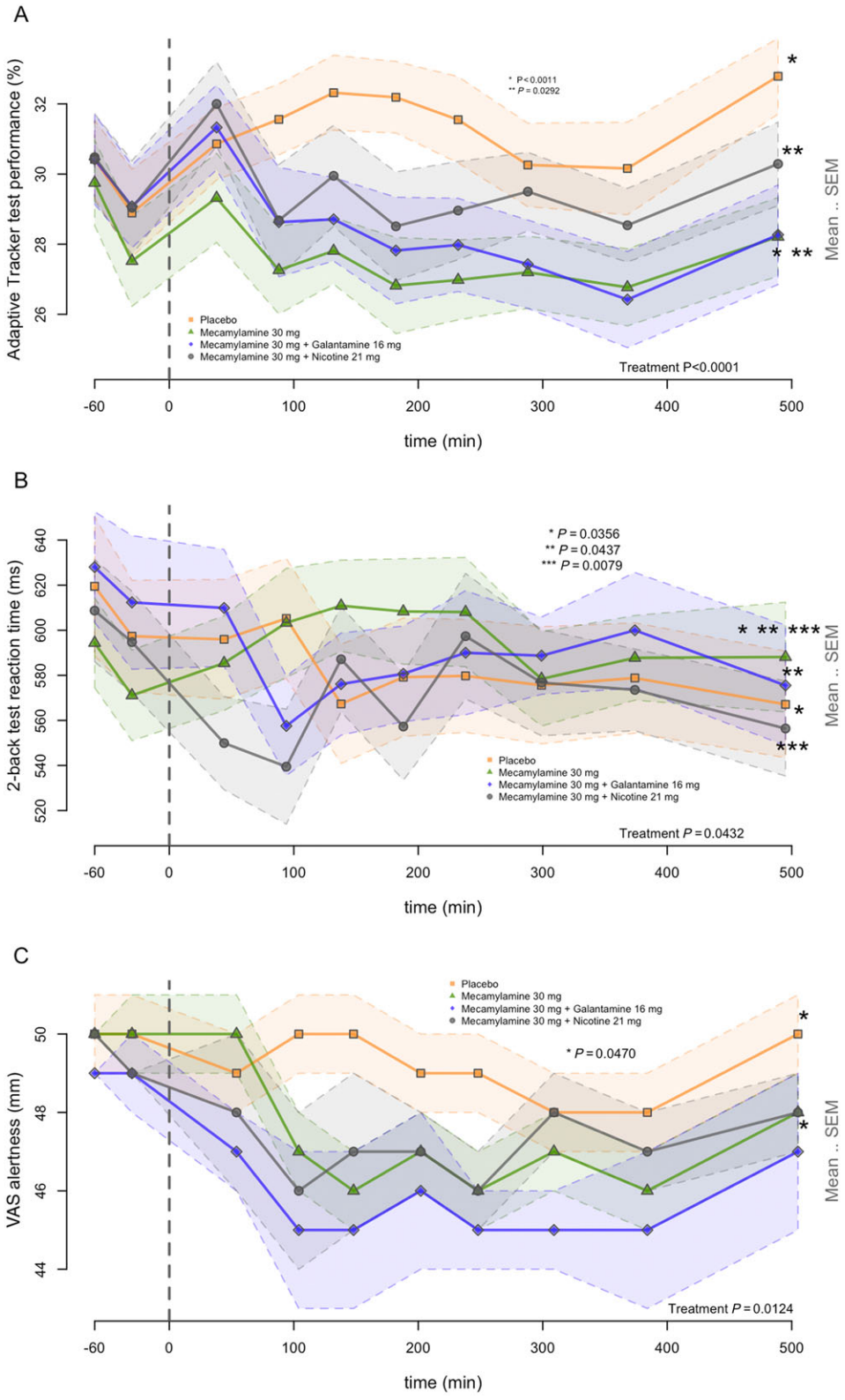

Figure 1

Effect on tests evaluating fine coordination, reaction time, attention and alertness.Mecamylamine, nicotine and galantamine effect *vs*. time during the adaptive tracking test (A), reaction time during the 2-back condition (B) and visual analogue scale evaluating alertness (C). Symbols represent the mean per treatment group and the polygon (shaded area around the mean) the standard error. Asterisks represent significance between groups (*P* value is mentioned per overall effect and per group, when applicable). Vertical discontinuous line represents time point zero

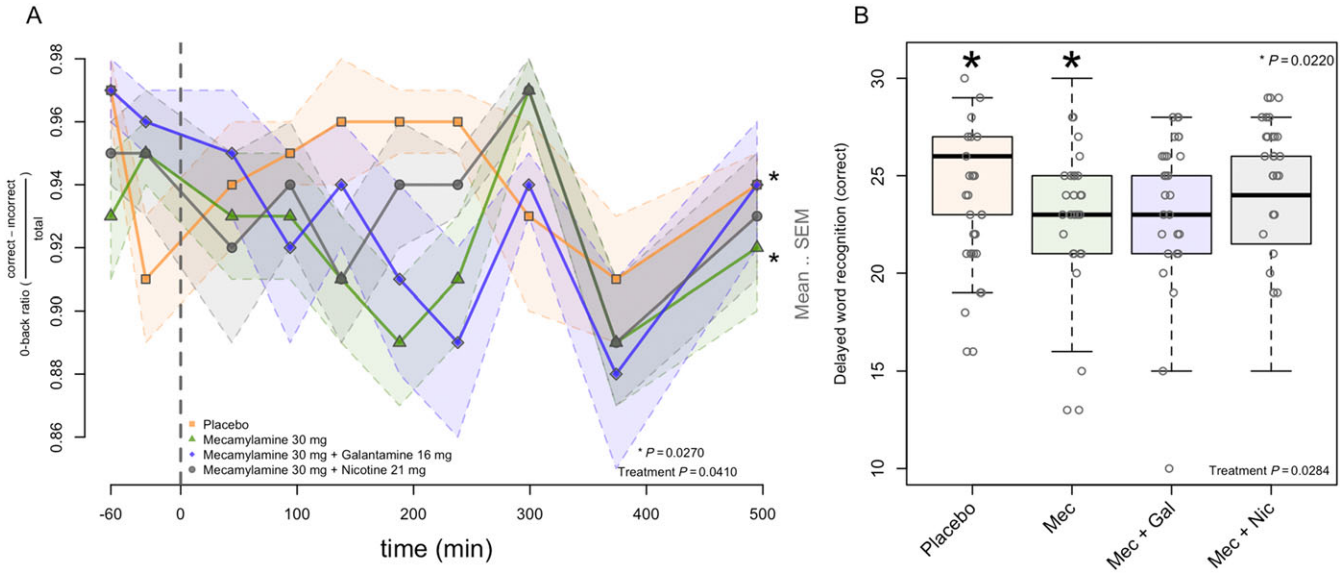

Figure 2

Effect on tests evaluating short and long-term retrieval. Mecamylamine, nicotine and galantamine effect *vs*. time during the 0-back condition ratio of correct–incorrect answers (A). Symbols represent the mean per treatment group and the polygon (shaded area around the mean) the standard error. Asterisks represent significance between groups (*P* value is mentioned per overall effect and per group, when applicable). Vertical discontinuous line represents time point zero. Asterisks represent significance between groups (*P* value is mentioned per treatment and per group, when applicable). Mecamylamine, nicotine and galantamine effect per treatment group during the delayed word recognition condition of the verbal visual learning test number of correct answers during the (B). The box plots represent the first and third quartile, the middle line the group mean and the vertical lines the confidence interval. Individual observations are plotted as well

difference: 36.0 ms, 95% confidence interval: –62.2 to –9.7, $P < 0.01$) and galantamine (mean difference: 27.2 ms, 95% confidence interval: –53.3 to –0.8, $P < 0.05$).

EEG. As shown in Figure 3A, the mean α power over Pz-Oz showed a significant overall effect ($P = 0.0132$); however, the only significant contrast was an increase of 14.9% (95% confidence interval: 6.0–24.6, $P < 0.005$) when nicotine was coadministrated with mecamylamine compared to mecamylamine (alone) administration (Table 1).

Mecamylamine showed a significant overall effect ($P = 0.0439$) on β power over the Pz-Oz leads. Mecamylamine administration reduced the β power by –7.1% (95% confidence interval: –13.7 to –0.1%, $P < 0.05$) when compared to placebo. Nicotine coadministration reversed mecamylamine effects by 10.7% (95% confidence interval: 2,9–19.1, $P < 0.01$; Figure 3).

Finger tapping. Mecamylamine showed a significant overall effect ($P < 0.0001$) on the finger tapping test and significantly decreased the mean number of taps recorded during the finger tapping test by –5.3 taps (95% confidence interval: –6.8 to –3.8, $P < 0.0001$).

VVLT. The only parameter from the VVLT where mecamylamine had a significant effect was on the number of correct answers during the delayed word recognition ($P = 0.0284$) condition. Mecamylamine administration caused more errors than placebo (–1.87 correct answers; 95% confidence interval: –3.46 to –0.28; $P = 0.02$; Figure 2B, Table 1).

VAS. A significant overall effect was observed on the mean VAS alertness (overall effect $P < 0.05$) and nausea ($P < 0.0001$) scores. Mecamylamine administration produced a significant decrease in the mean subjective feeling of alertness of –1.82 mm (95% confidence interval: –3.61 to –0.02, $P < 0.05$; Figure 1C). Mecamylamine plus galantamine increased the mean VAS nausea measurement 90% (95% confidence interval: 47–146%, $P < 0.0001$; back-transformed), and in combination with nicotine caused an increase of 53% (95% confidence interval: 19–98%, $P < 0.005$; back-transformed) compared to mecamylamine alone.

Physiological measures

Vital signs. Examination of the mean standing systolic blood pressure (SBP) showed a significant overall effect ($P < 0.005$). While mecamylamine nonsignificantly decreased the mean standing SBP by –5.3 mmHg, nicotine coadministration produced an additional decrease of –8.8 mmHg (95% confidence interval: –16.1 to –1.6, $P < 0.05$) when compared to mecamylamine alone (Table S1). A significant overall effect for heart rate in both standing and supine positions ($P < 0.0001$) was observed. Mecamylamine administration produced an increase in heart rate in supine (mean 12.3 beats min^{-1}, 95% confidence interval: 9.7–14.9, $P < 0.0001$) and standing (mean 26.7 beats min^{-1}, 95% confidence interval: 19.7–33.8] $P < 0.0001$) positions. Coadministration of nicotine and galantamine did not

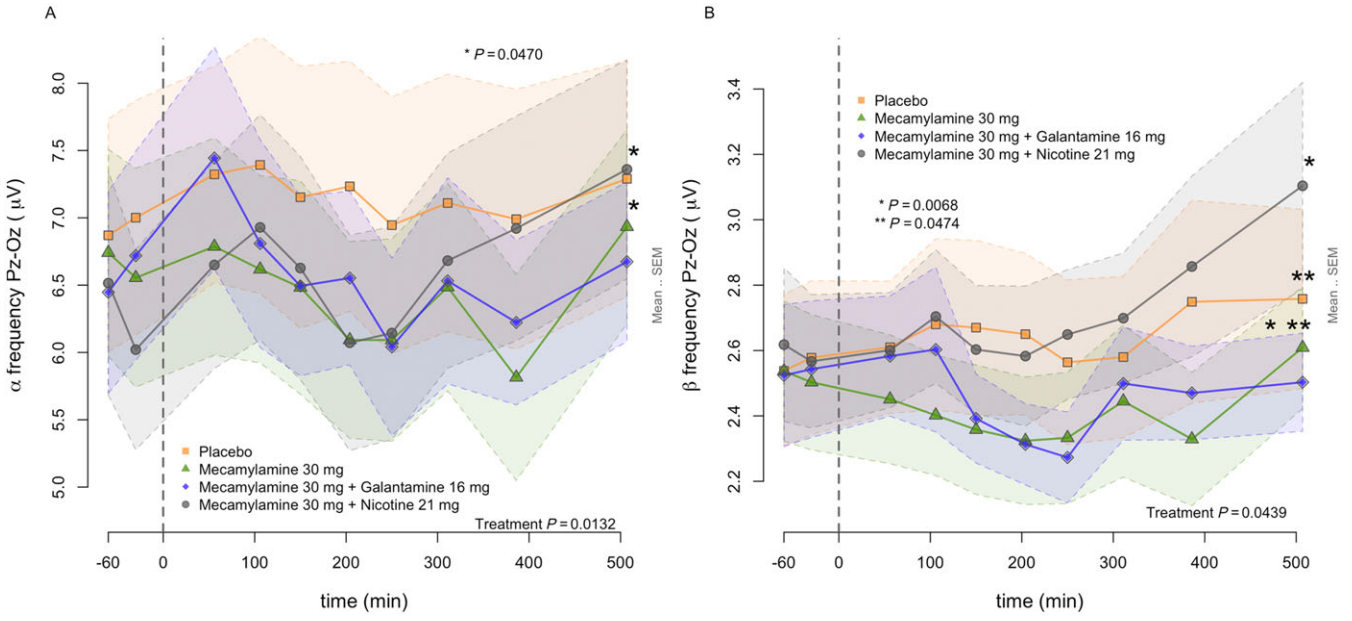

Figure 3

Effect on the electroencephalogram. Mecamylamine, nicotine and galantamine effect *vs.* time for the electroencephalogram Pz-Oz α (A) and Pz-Oz β (B) frequency. Symbols represent the mean per treatment group and the polygon the standard error around the mean. Asterisks represent significance between groups (*P* value is mentioned per treatment and per group, when applicable). The vertical discontinuous line represents time point zero

influence the heart rate significantly. There were no changes in the body temperature in any of the groups compared to placebo.

There were no clinically significant changes in values for haematology, chemistry and urinalysis parameters.

AEs. AEs were less frequently reported in the placebo group (46.4%), followed by the galantamine (89.3%), nicotine (89.7%) and finally the mecamylamine (93.1%) group (Table 2). No severe or serious AEs were reported. Whenever due to AEs subjects decided to stop their participation a note was recorded. Subjects were allowed to resume their participation once the symptoms decreased to an acceptable level to assure that the AE had no influence on the tests.

Discussion

In this cross-over study we investigated the cognitive and neurophysiological effects of administration of 30 mg of the nicotinic acetylcholine receptor antagonist mecamylamine in healthy subjects, and its potential reversibility by coadministration of a nicotinic agonist or a cholinesterase inhibitor. Administration of mecamylamine showed a consistent pattern of worse performance on the neuropsychological and neurophysiological tests when compared to placebo. In addition, the coadministration of transdermal nicotine (21 mg) caused reversal of the mecamylamine effect in tests evaluating fine motor coordination and reaction time. Nicotine also reversed mecamylamine effects on the α and β frequencies of the EEG. The effects of coadministration of galantamine were less clear and reversal was only seen in reaction time during the N-back test tests and on the β frequency of the EEG.

Administration of 30 mg mecamylamine resulted in a decrease in performance of tests evaluating attention, motor fluency, visuo- (fine) motor coordination, short-term memory, sustained attention and reaction time. In a previous study we investigated the effects of 10 and 20 mg mecamylamine as a single administration in healthy young subjects, which showed poorer performance on the same tests but no increase in reaction time. A direct comparison to scopolamine (0.5 mg as a 15 min infusion) showed that the adverse cognitive effects of this nonselective muscarinic receptor agonist were larger compared to both dose levels of mecamylamine. The magnitude of the changes in all test paradigms and observed in the current study with 30 mg mecamylamine was larger compared to administrating lower mecamylamine doses. Where scopolamine nonselectively antagonizes muscarinic ACh receptors [6] mecamylamine has been shown *in vitro* to noncompetitively antagonize the most important central nicotinic receptors [35]. Nicotinic activation is associated with changes in visuospatial and declarative memory, decision-making processes, integration of acquired stimuli, fine motor skills and learning [36], which are consistent with the measured mecamylamine induced effects as a result of central nAChR blockade in the current study. Scopolamine, the traditionally most widely used cognitive challenge, has a sedative effect [15], which can be expected to contribute to the cognitive deficits that scopolamine administration induces, and it may not be possible to differentiate the contribution of sedation to the cognitive deficits. This may be a limitation of the use of

Table 2

Summary of number of subjects with an adverse event and number of adverse events with the highest incidence in descending order of incidence

	Mecamylamine (*n* = 29)		Mecamylamine + galantamine (*n* = 28)		Mecamylamine + nicotine (*n* = 29)		Placebo (*n* = 28)	
Adverse event	**Number of events**	**Number of subjects (%)**	**Number of events**	**Number of subjects (%)**	**Number of events**	**Number of subjects (%)**	**Number of events**	**Number of subjects (%)**
All events	76	27 (93.1)	101	25 (89.3)	108	26 (89.7)	26	13 (46.4)
Nausea	3	3 (10.3)	15	14 (50.0)	12	12 (41.4)	-	-
Somnolence	14	10 (34.5)	12	12 (42.9)	11	10 (34.5)	1	1 (3.6)
Dizziness	5	5 (17.2)	13	11 (39.3)	13	11 (37.9)	1	1 (3.6)
Fatigue	8	7 (24.1)	8	8 (28.6)	6	6 (20.7)	4	4 (14.3)
Orthostatic hypotension	8	8 (27.6)	4	4 (14.3)	5	5 (17.2)	6	4 (14.3)
Headache	3	3 (10.3)	3	3 (10.7)	6	6 (20.7)	4	2 (7.1)
Application site vpruritus	1	1 (3.4)	-	-	7	6 (20.7)	1	1 (3.6)
Ocular hyperaemia	3	2 (6.9)	2	2 (7.1)	6	6 (20.7)	-	-
Vision blurred	6	5 (17.2)	1	1 (3.6)	4	4 (13.8)	-	-
Constipation	5	4 (13.8)	2	2 (7.1)	5	5 (17.2)	-	-
Vomiting	1	1 (3.4)	3	3 (10.7)	4	4 (13.8)	-	-
Dizziness postural	2	1 (3.4)	1	1 (3.6)	3	3 (10.3)	-	-
Abdominal pain	3	3 (10.3)	3	3 (10.7)	2	2 (6.9)	-	-
Feeling abnormal[a]	1	1 (3.4)	3	3 (10.7)	-	-	2	2 (7.1)
Abdominal distension	1	1 (3.4)	3	3 (10.7)	-	-	-	-

[a]Feeling abnormal was used by the research physician when no other symptom could describe the feeling the subject was experiencing.

the scopolamine model as an early proof-of-pharmacology tool for compounds with a suspected procognitive effect. Moreover, for proof-of-pharmacology of nicotinic compounds, it would make more sense to use a nicotinic rather than a muscarinic antagonist, even though the nicotinic and muscarinic neuronal systems are intimately linked [37–39]. In previous studies scopolamine 0.5 mg induced in healthy subjects a higher incidence of somnolence (24.0–58.3%; unpublished data) and dizziness (48.0–76.9%; unpublished data) when compared to mecamylamine 30 mg (dizziness 17.2 and somnolence 34.5%) as shown in this study in Table 2. The most frequent AEs after mecamylamine administration were fatigue (24.1%) and orthostatic hypotension (27.6%). The decrease in attention after mecamylamine administration might suggest that it is not due to sedation (as with muscarinic antagonists) but to impairment of attention/concentration due to mecamylamine, suggesting that mecamylamine as challenge drug might be preferred to induce cognitive impairment with fewer sedative effects. Donepezil 5 mg has been reported as the only drug that partially reversed the effects induced by mecamylamine 20 mg in healthy subjects, which consisted of slowing of the inspection time during visual discrimination [12]. Similar to our study in humans, mecamylamine-induced cognitive effects were significantly reversed by nicotine in mice. In this animal study, however, nicotine did not reverse scopolamine induced effects [40]. While numerous groups have been able to demonstrate reversal of scopolamine effects by coadministration of compounds with nAChR agonist activity in animal models, none of these results were ever reproduced in humans with the mecamylamine challenge model. The proposed mecamylamine model therefore seems superior to the scopolamine challenge model to use in translational and early phase clinical drug studies investigating novel nicotinic agonists.

To our knowledge, reversal of mecamylamine-induced effects by a nAChR agonist has not been previously demonstrated in humans. In this study, we provide evidence that coadministration of 21 mg of transdermal nicotine partially reversed the cognitive effects on tests evaluating visuo- (fine) motor coordination, short- and long-term memory, and reaction time observed following mecamylamine administration. Coadministration of nicotine also appeared to reverse mecamylamine effects in tests evaluating alertness and visuospatial memory, but these effects were not significant. Mecamylamine is a nicotinic competitive antagonist that *in vitro* completely blocks the effect of nicotine on several nAChRs [41]. *In vivo* reversal by nicotine of the cognitive effects resulting from mecamylamine administration suggests that both drugs affect the same system, namely the nicotinic

cholinergic central neuronal system; however, the mechanism is barely understood as the receptor site of action of mecamylamine (a noncompetitive nAChR antagonist) differs from that of nicotine (being a competitive agonist). The current study provides evidence of reversibility of the mecamylamine effects; however, to determine the competitive effect–concentration relationship between nicotine and mecamylamine, a range of nicotine doses should be explored to better elucidate this relationship *in vivo* and determine if the extent of the reversal can be complete if a sufficiently high dose is chosen. Lack of reversal of mecamylamine effects occurred in tests evaluating motor fluency (finger taps), sustained attention (ratio of the 0-back paradigm), and working memory (delayed word recognition of the VVLT) and alertness (measured by VAS). One possible explanation to the fact that mecamylamine induced deficits in the majority but not all cognitive tests and furthermore, nicotine reversed mecamylamine effects in some of these, might be related to mecamylamine different affinity and dissociation time measured *in vitro* to each nAChR subtype, which might explain differences in effects observed in the cognitive tests. Mecamylamine is a noncompetitive antagonist that is believed to have a different binding site on the nicotine receptor when compared to nicotine. Recent experiments using a sequence exchange to decrease receptor inhibition by mecamylamine show that this receptor affinity also decreases and produces a comparable potentiation of long-term inhibition by nicotine; therefore, the activity profile of nicotine for the various neuronal AChRs seems influenced by subtype specific competitive and noncompetitive effects [42]. Although we deliberately enrolled sporadic smokers in the study to avoid nausea due to administration of nicotine 21 mg (the approved starting dose for patients consuming more than 10 cigarettes a day and willing to abstain from smoking) a high incidence of nausea and vomiting was still observed, probably because we included subjects who smoked fewer than five cigarettes per day aiming to test the cognitive effects of nicotinic compounds in healthy subjects, and not the effects of withholding nicotine in nicotine-dependent subjects, while inducing as few AEs due to nicotine as possible. Nicotine induced nausea may, however, have negatively influenced performance on the cognitive tests. A limitation to this study is the absence of cotinine levels to confirm that subjects enrolled in the study would be indeed sporadic smokers. Coadministration of a nicotinic agonist with different activity, i.e. selective $\alpha_3\beta_4$, α_7 or $\alpha_4\beta_2$ agonists, can be expected to produce different profiles in the different cognitive areas in humans.

While galantamine appeared to reverse mecamylamine induced cognitive effects, the differences with placebo were not significant except for reaction time during the 2-back condition. Galantamine exerts a dual mode of action, namely cholinesterase inhibitory effect and allosterically potentiating ligand of the nicotinic AChR *in vitro*, which other acetylcholinesterase inhibitors such as donepezil lack (Maelicke et al. 2001). Galantamine has been reported to partially reverse electroencephalographic and sedative disturbances produced by scopolamine. In our study, mecamylamine effects on EEG were not affected by administration of 16 mg of galantamine. One important difference between the two studies is that in the scopolamine study a galantamine dose of 0.5 mg kg^{-1} was used [43], while in the current study the dose was on average 0.21 mg kg^{-1}. We expected that the reversal of a nicotinic antagonist (mecamylamine) by a nicotinic allosteric modulator as galantamine would require a lower concentration range than reversal of a muscarinic antagonist (scopolamine). From clinical use, it is known that CEIs have to be administered in high doses to cause symptomatic relief of cognitive deficits in AD [44]. The lack of significant results in our study could be caused by an insufficiently high dose. This idea is supported by the trends observed in the galantamine results, albeit nonsignificant but almost all resulted in reversal of mecamylamine effects. Even though a higher galantamine dose in this study was considered, the expected side effects (severe nausea and vomiting) in healthy subjects after an acute administration of galantamine was an important argument not to administer higher doses of galantamine. In retrospect, this was the right decision, as in this study there was already a high incidence of AEs related to the mechanism of action of the drug (see Table 2). Figure 2A shows the ratio graphed *vs*. time of the 0-back test paradigm. Time-points between 300 and 400 min show an initial steep increase and a following decrease in the groups treated with mecamylamine. Whether the outlier was consequence of a decline in mecamylamine effects (T_{MAX} = 180 min), or the result of an *after lunch dip* (approximately at 350 min) is difficult to defend since it was only observed in this one test.

Mecamylamine produced a decrease in β frequency power in the posterior bipolar leads of the surface EEG, and led to a nonsignificant decrease in α power as well as an increase in θ power, which correspond to reports from previous studies with mecamylamine [45]. A decrease in posterior α power and an increase in frontal and posterior θ power has also been observed in patients with AD [46]. Nicotine significantly diminished the decrease in α and β power induced by mecamylamine in the posterior leads of the EEG, mainly at the last time points (>300 min), producing an even greater increase when compared to placebo. The T_{MAX} during transdermal nicotine patch administration is reported at 6 h (360 min), consistent with the time where the maximum effect was observed in the EEG [17]. The increase of the β power at the end of the trial observed in the EEG could be explained by a difference in the T_{MAX}, of mecamylamine and nicotine.

Administration of a single dose of 30 mg of mecamylamine was safe, and generally tolerated well enough for a challenge model involving cognitive testing. The most common AEs in the groups receiving either mecamylamine alone or mecamylamine in combination with galantamine or nicotine were symptoms related to nicotinic gastrointestinal and CNS symptoms. Nausea and vomiting were the most frequently reported AEs on occasions where nicotine and galantamine were coadministered. It could be postulated that the mechanism for the nausea and vomiting is related to the high density of α_3, α_4, β_2, and, to a lesser extent, α_5 and β_4 nAChRs in the area postrema [47]. Blockage of the sympathetic system by mecamylamine and its effects on the BP has been extensively studied and described before in patients with hypertension, but not in healthy subjects [48].

Mecamylamine effect on BP in healthy subjects mainly impaired the compensatory mechanisms, inducing orthostatic hypotension. The effects of mecamylamine on the blood pressure have been further studied using a pharmacokinetic and pharmacodynamic model [49].

In conclusion, we have confirmed in humans that a single dose of mecamylamine 30 mg induces a significant disturbance in cognitive functions such as visual (fine) motor coordination, sustained attention, short- and long-term memory, reaction time, and changes in the EEG (decrease in α and in β power), and that these effects could be partially reversed by the coadministration of nicotine. This suggests that the mecamylamine challenge model can be used for proof-of-pharmacology studies nAChR agonists in humans, providing a useful tool in drug development of cognition enhancing compounds currently being developed to treat AD and schizophrenia, among other diseases.

Competing interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare to have no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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References

1 Jones S, Sudweeks S, Yakel JL. Nicotinic receptors in the brain: correlating physiology with function. Trends Neurosci 1999; 22: 555–61.

2 Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. Psychopharmacology (Berl) 2006; 184: 523–39.

3 Court J, Piggott M, Lloyd S, Cookson N, Ballard C, McKeith I, *et al*. Nicotine binding in human striatum: elevation in schizophrenia and reductions in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease and in relation to neuroleptic medication. Neuroscience 2000; 98: 79–87.

4 Parri HR, Hernandez CM, Dineley KT. Research update: alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. Biochem Pharmacol 2011; 82: 931–42.

5 Ebert U, Kirch W. Scopolamine model of dementia: electroencephalogram findings and cognitive performance. Eur J Clin Invest 1998; 28: 944–9.

6 Ali-Melkkilä T, Kanto J, Iisalo E. Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. Acta Anaesthesiol Scand 1993; 37: 633–42.

7 Beinat C, Banister SD, Herrera M, Law V, Kassiou M. The therapeutic potential of α7 nicotinic acetylcholine receptor (α7 nAChR) agonists for the treatment of the cognitive deficits associated with schizophrenia. CNS Drugs 2015; 29: 529–42.

8 Vallés AS, Borroni MV, Barrantes FJ. Targeting brain α7 nicotinic acetylcholine receptors in Alzheimer's disease: rationale and current status. CNS Drugs 2014; 28: 975–87.

9 Webster JC, Francis MM, Porter JK, Robinson G, Stokes C, Horenstein B, *et al*. Antagonist activities of mecamylamine and nicotine show reciprocal dependence on beta subunit sequence in the second transmembrane domain. Br J Pharmacol 1999; 127: 1337–48.

10 Newhouse PA, Potter A, Corwin J, Lenox R. Age-related effects of the nicotinic antagonist mecamylamine on cognition and behavior. Neuropsychopharmacology 1994; 10: 93–107.

11 Newhouse PA, Potter A, Corwin J, Lenox R. Acute nicotinic blockade produces cognitive impairment in normal humans. Psychopharmacology (Berl) 1992; 108: 480–4.

12 Thompson JC, Stough C, Ames D, Ritchie C, Nathan PJ. Effects of the nicotinic antagonist mecamylamine on inspection time. Psychopharmacology (Berl) 2000; 150: 117–9.

13 Brucato FH, Levin ED, Rose JE, Swartzwelder HS. Intracerebroventricular nicotine and mecamylamine alter radial-arm maze performance in rats. Drug Dev Res 1994; 31: 18–23.

14 Woodruff-Pak DS. Mecamylamine reversal by nicotine and by a partial α7 nicotinic acetylcholine receptor agonist (GTS-21) in rabbits tested with delay eyeblink classical conditioning. Behav Brain Res 2003; 143: 159–67.

15 Baakman AC, Alvarez-Jimenez R, Rissmann R, Klaassen ES, Stevens J, Goulooze SC, *et al*. An anti-nicotinic cognitive challenge model using mecamylamine in comparison with the anti-muscarinic cognitive challenge using scopolamine. Br J Clin Pharmacol 2017; 83: 1676–87.

16 Kaufmann H. Consensus statement on the definition of orthostatic hypotension, pure autonomic failure and multiple system atrophy. Clin Auton Res 1996; 6: 125–6.

17 DeVeaugh-Geiss AM, Chen LH, Kotler ML, Ramsay LR, Durcan MJ. Pharmacokinetic comparison of two nicotine transdermal systems, a 21-mg/24-hour patch and a 25-mg/16-hour patch: a randomized, open-label, single-dose, two-way crossover study in adult smokers. Clin Ther 2010; 32: 1140–8.

18 Riemann D, Gann H, Dressing H, Müller WE, Aldenhoff JB. Influence of the cholinesterase inhibitor galanthamine hydrobromide on normal sleep. Psychiatry Res 1994; 51: 253–67.

19 Maelicke A, Albuquerque EX. Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease. Eur J Pharmacol 2000; 393: 165–70.

20 Coyle J, Kershaw P. Galantamine, a cholinesterase inhibitor that allosterically modulates nicotinic receptors: effects on the course of Alzheimer's disease. Biol Psychiatry 2001; 49: 289–99.

21 Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, *et al*. Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. Biol Psychiatry 2001; 49: 279–88.

22 Lim HK, Juh R, Pae CU, Lee BT, Yoo SS, Ryu SH, *et al*. Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. Neuropsychobiology 2008; 57: 181–7.

23 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 1984; 18 (Suppl 1): 69S–72S.

24 van Steveninck AL, Schoemaker HC, Pieters MSM, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis, and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172–80.

25 Andrew JM. Delinquents and the tapping test. J Clin Psychol 1977; 33: 786–91.

26 Liem-Moolenaar M, Zoethout RWM, de Boer P, Schmidt M, de Kam ML, Cohen AF, *et al*. The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. J Psychopharmacol 2010; 24: 1671–9.

27 Wezenberg E, Sabbe BGC, Hulstijn W, Ruigt GSF, Verkes RJ. The role of sedation tests in identifying sedative drug effects in healthy volunteers and their power to dissociate sedative-related impairments from memory dysfunctions. J Psychopharmacol 2007; 21: 579–87.

28 Schmitt JAJ, Wingen M, Ramaekers JG, Evers EAT, Riedel WJ. Serotonin and human cognitive performance. Curr Pharm Des 2006; 12: 2473–86.

29 Zuurman L, Passier PC, de Kam ML, Kleijn HJ, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of the intravenous CB1 receptor agonist Org 26828 in healthy male volunteers. J Psychopharmacol 2010; 24: 1689–96.

30 Milner B. Visually-guided maze learning in man: effects of bilateral hippocampal, bilateral frontal, and unilateral cerebral lesions. Neuropsychologia 1965; 3: 317–38.

31 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211–8.

32 Twa MD, Bailey MD, Hayes J, Bullimore M. Estimation of pupil size by digital photography. J Cataract Refract Surg 2004; 30: 381–9.

33 Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S, *et al*. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 2017; 46: D1091–106.

34 Alexander SPH, Peters JA, Kelly E,, Marrion NV, Faccenda E, Harding SD, *et al*. The Concise Guide to PHARMACOLOGY 2017/18: Ligand-gated ion channels. Br J Pharmacol 2017; 174 (Suppl): S130–59.

35 Papke RL, Sanberg PR, Shytle RD. Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. J Pharmacol Exp Ther 2001; 297: 646–56.

36 Burgess N, Maguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. Neuron 2002; 35: 625–41.

37 Levin ED, Rose JE, McGurk SR, Butcher LL. Characterization of the cognitive effects of combined muscarinic and nicotinic blockade. Behav Neural Biol 1990; 53: 103–12.

38 Levin ED, McGurk SR, South D, Butcher LL. Effects of combined muscarinic and nicotinic blockade on choice accuracy in the radial-arm maze. Behav Neural Biol 1989; 51: 270–7.

39 Levin ED, Rose JE. Nicotinic and muscarinic interactions and choice accuracy in the radial-arm maze. Brain Res Bull 1991; 27: 125–8.

40 Levin ED, Kaplan S, Boardman A. Acute nicotine interactions with nicotinic and muscarinic antagonists: working and reference memory effects in the 16-arm radial maze. Behav Pharmacol 1997; 8: 236–42.

41 Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev 2009; 89: 73–120.

42 Webster JC, Francis MM, Porter JK, Robinson G, Stokes C, Horenstein B, *et al*. Antagonist activities of mecamylamine and nicotine show reciprocal dependence on beta subunit sequence in the second transmembrane domain. Br J Pharmacol 1999; 127: 1337–48.

43 Baraka A, Harik S. Reversal of central anticholinergic syndrome by galanthamine. JAMA 1977; 238: 2293–4.

44 Olin J, Schneider L. Galantamine for Alzheimer's disease. Cochrane Database Syst Rev 2002; (3): CD001747.

45 Pickworth WB, Fant RV, Butschky MF, Henningfield JE. Effects of mecamylamine on spontaneous EEG and performance in smokers and non-smokers. Pharmacol Biochem Behav 1997; 56: 181–7.

46 van Straaten EC, Scheltens P, Gouw AA, Stam CJ. Eyes-closed task-free electroencephalography in clinical trials for Alzheimer's disease: an emerging method based upon brain dynamics. Alzheimers Res Ther 2014; 6: 86.

47 Léna C, Changeux JP. Pathological mutations of nicotinic receptors and nicotine-based therapies for brain disorders. Curr Opin Neurobiol 1997; 7: 674–82.

48 Ford RV, Madison JC, Moyer JH. Pharmacology of mecamylamine. Am J Med Sci 1956; 232: 129–43.

49 Alvarez-Jimenez R, Baakman AC, Stevens J, Goulooze SC, Hart EP, Rissmann R, *et al*. Pharmacokinetics and pharmacodynamics of oral mecamylamine – development of a nicotinic acetylcholine receptor antagonist cognitive challenge test using modelling and simulation. J Psychopharmacol 2017; 31: 192–203.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

http://onlinelibrary.wiley.com/doi/10.1111/bcp.13507/suppinfo

Table S1 Mean differences (contraststst) and least squared (LS) means per treatment group on the neurophysiological and physiological parameters. F(NumDF, DenDF) = F-value

Table S2 Medicinal products, dose and administration route per treatment arm